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TITLE: Development of Methods for the Real-Time and Rapid Identification and Detection of TSE in Living Animals Using Fluorescence Spectroscopy of the Eye

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14. ABSTRACT

Our investigations continue to suggest that the most promising part of the eye for revealing spectroscopic signatures of neurological disease is the retina. Our experiments have so far been limited to sheep. Our experiments have been designed to address the following questions:

1. Can ocular spectra be diagnostic of neurological disease?
2. Can the effects of neurological disease be separated from those associated with normal aging?
3. Can images of the eye be obtained that report on neurological disease?

Our results indicate that we can answer all three questions in the affirmative. Enormous progress has been made since the inception of this work. We are continuing to search funding for its continuation and are currently implementing methods to make the scanning process more rapid.

15. SUBJECT TERMS
TSE, fluorescence spectroscopy, real time, eye, diagnostics

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INTRODUCTION:

Transmissible spongiform encephalopathies (TSEs) are thought to be caused by the accumulation of abnormal protease-resistant proteins called prions, which are found in aging central nervous system tissue and in the eyes. Other protease-resistant compounds, collectively called lipofuscins, also accumulate in CNS. Lipofuscins accumulate in the eye, especially in the diseased eye. An increase in lipofuscin accumulation is known to occur in human Creutzfeldt-Jakob disease victims and in other cases of experimental TSEs. Lipofuscins are fluorescent compounds with characteristic optical spectra. Some individual lipofuscin compounds (especially from the eye) have been studied in detail with regard to optical and chemical properties. The spinal cord and brain also have been observed to be fluorescent under certain wavelengths of light. This is due in part to lipofuscin accumulation in this tissue. The literature indicates that abnormal TSE prions also display characteristic optical spectra. The Principal Investigator's (PI's) preliminary data indicate that the fluorescent spectra of scrapie-infected sheep brain differ substantially from that of the noninfected sheep brain. The purpose of this study is to test the hypothesis that this spectral difference is the result of altered lipofuscin and/or prion spectral properties. Lipofuscins and prions may serve as useful fluorescent markers, which are correlated with the occurrence of TSEs and can be detected by spectroscopy.

BODY: KEY RESEARCH ACCOMPLISHMENTS:

During the first year of this study, we made only marginal progress as a result of difficulties in transmittal of funds to a collaborating laboratory. We dissected sheep and cow eyes and performed fluorescence spectroscopy on all the major eye components and reports that the cornea, lens, retina, and optic nerve show promise. Of these, the optic nerve showed the most potential for changes in spectral properties as a result of prion disease. Unfortunately, because of the lack of control tissues, the only conclusion that can be drawn is that the optic nerve shows the most intense fluorescence. The first year of this project, however, suffered from several setbacks: namely, the inability to transfer funding efficiently to the USDA collaborators and the difficulty of obtaining proper tissue samples. For example, in year one, we were forced to work under the unsatisfactory circumstances of comparing spectra from scrapie-infected sheep eyes with those from healthy cow eyes.

Year two had shown modest improvements in our working conditions. Funds were finally able to be transferred to the USDA collaborators and we were able to establish spectral comparisons between healthy and scrapie infected sheep eyes. The extent of our sampling was not, however, as large as we would like it to be and more importantly, the tissues were not age matched.

In year three considerable progress was made. Our preliminary investigations so far suggest that the most promising part of the eye for revealing spectroscopic signatures of neurological disease is the retina. Our experiments have been limited to sheep. Our experiments have been designed to address the following questions:

1. Can ocular spectra be diagnostic of neurological disease?
2. Can the effects of neurological disease be separated from those associated with normal aging?
3. Can images of the eye be obtained that report on neurological disease?

As indicated in the previous report, all of these questions have been answered in the affirmative and compelling data have been presented to this effect.

In the course of this work, we have also investigated whether CNS tissue can be detected in meat products. The removal of Central Nervous System (CNS) tissues as part of Bovine Spongiform

Encephalopathy (BSE) risk material is one of the highest priority tasks to avoid contamination of the human food chain with BSE. No currently available method enables the real-time detection of possible CNS tissue contamination on carcasses during slaughter. Lipofuscin fluorescence was investigated as a marker for real-time detection of CNS contamination. Front-faced fluorescence spectra of brain and spinal cord samples from 11 cattle gave identical, reproducible fluorescence signal patterns with high intensities. The specificity of these spectra was assessed investigating 13 different non-CNS tissues enabling the differentiation of brain and spinal cord by signal intensity and structure of the spectra, respectively. Small quantities of bovine spinal cord were reliably detected in the presence of raw bovine skeletal muscle, fat, and vertebrae. These data may form the fundamental basis for the development of a prototype device allowing real-time monitoring of CNS tissue contamination on bovine carcasses and meat cuts.

Lastly, we discovered, much to our disappointment, that room temperature ionic liquids, which are capable of dissolving most substances, are incapable of dissolving lipofuscin. This conclusion is limited to phosphonium and imidazolium based liquids.

REPORTABLE OUTCOMES:

For this proposal, the reportable outcomes are as follows.

- Ms. Tessa Calhoun received her B.S. in chemistry in Spring 2005 and will be entering graduate school in chemistry at the University of California at Berkeley.
- Ms. Erin Campbell received her B.S. in biochemistry in Spring 2005 and will be taking a year off before applying to graduate schools.
- Dr. G. Krishnamoorthy is now Assistant Professor, Department of Chemistry, Indian Institute of Technology, Guwahati, Assam, India.
- Ms. Alyse Hurd, an undergraduate student who has worked on this project, is applying to graduate schools in chemistry and has already been accepted to the programs at the University of Arizona and UCLA.

We have submitted an invention disclosure based upon on work, and are currently working on methods to make the microscopic scanning process described above more rapid.

CONCLUSIONS:

The major conclusions of the work executed so far are that specific parts of sheep eyes have been identified that may provide spectroscopic signatures of prion disease: these are the retina, lens, and sclera. Surprisingly, the optic nerve did not provide spectroscopic differences between healthy and infected tissue, as was anticipated in the year 1 report. All parts of the eye have been investigated. We note, however, that the samples are not age matched. Given the reproducibility of the spectral features for retina, lens, and sclera (presented in earlier reports), this may prove to be a positive aspect since our data demonstrate the increase of autofluorescence from eyes as a function of age. Specific wavelengths have been identified for exciting and detecting useful fluorescence signatures. The spectroscopic signatures of lipofuscin enable it to discriminate CNS tissue in meat products with as much sensitivity as PCR.

APPENDICES:

None.